

Broad CD8⁺ T cell cross-recognition of distinct influenza A strains in humans

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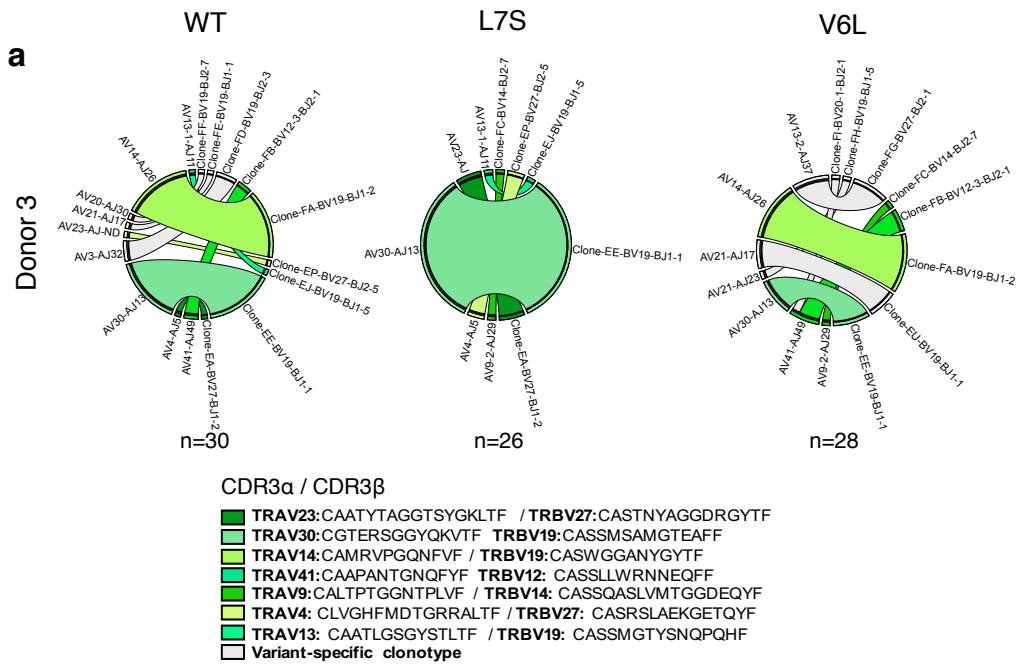
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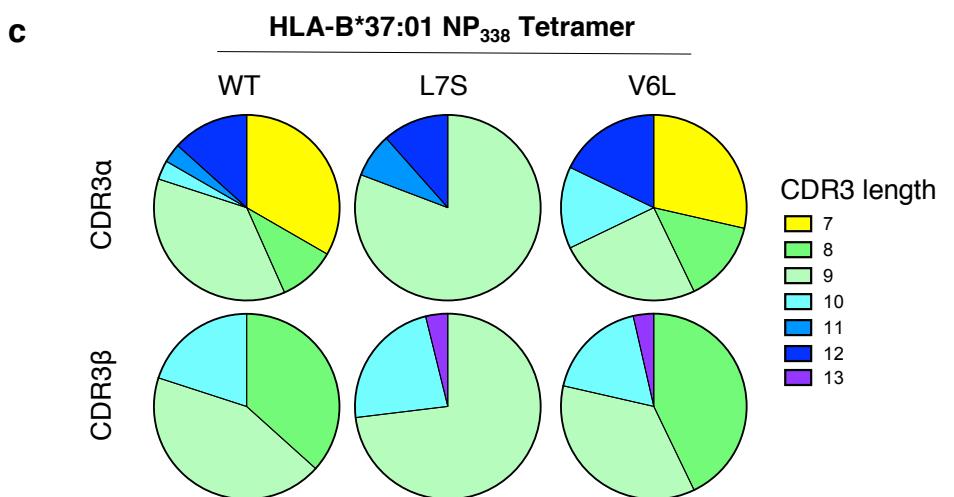
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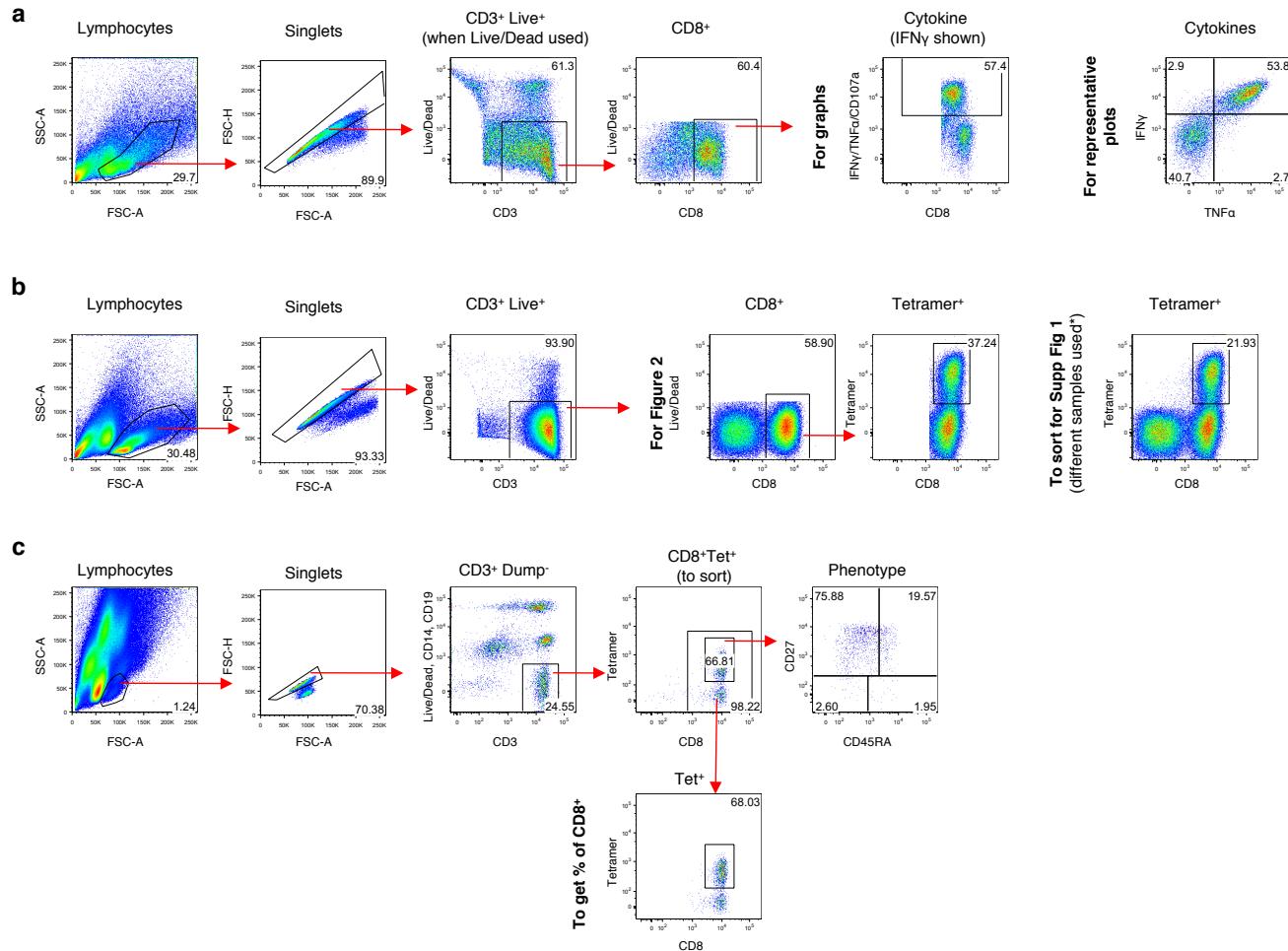
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Code	TRAV	TRAJ	CDR3 α	Length	TRBV	TRBJ	CDR3 β	Length	Frequency		
									WT	L7S	V6L
EE	30	13	CGTERSGGYQKVTF	9	19	1-1	CASSMSAMGTEAFF	9	33	73	18
FA	14	26	CAMRVPQNQFV	7	19	1-2	CASWGGANYGYTF	8	33		29
FB	41	49	CAAPANTGNQFYF	88	12-3	2-1	CASSLLWRNNEQFFF	9	7		11
FC	9-2	29	CALPTPGGNTPLVF	9	14	2-7	CASSQASLVMTGGDEQYF	13		4	4
EA	23		CAATYTAGGTSYGKLT	12	27	1-2	CASTNYAGGDRGYTF	10	3	12	
EP	4	5	CLVGHFMDTGRRALTF	11	27	2-5	CASRSLAEKGETQYF	10	3	8	
EJ	13-1	11	CAATLGSGYSTLTF	9	19	1-5	CASSMGTYSNQPQHF	10	3	4	
FD	3	32	CAVRDDLWGGAATNKLF	12	19	2-3	CASSYGTGSADTQYF	10	10		
FE	21	17	CADLPIKAAGNKLTF	10	19	1-1	CASSGVRATEAFF	8	3		
FF	20	30	CAVMVNRRDDKIF	8	19	2-7	CASSIGQAYYEQYF	9	3		
FG	13-2	37	CAESYMGSSNTGKLIF	12	27	2-1	CASSSLMGGTDEQFFF	10			18
EU	21	17	CAVLPIKAAGNKLTF	10	19	1-1	CASSGVRAEFFECT	8			14
FH	21	23	CAVMIYNQGGKLIF	9	19	1-5	CASKGDSPHQPKQHF	9			4
FI	41	49	CAAPANTGNQFYF	8	20-1	2-1	CSATGTSGYNEQFFF	9			4
								Total # of sequences	30	26	28
								Frequency of cross-reactivity	83	100	61



Supplementary Figure 1. Cross-recognition of NP₃₃₈ variants after *in vitro* amplification

PBMCs from HLA-B*37:01⁺ Donor 3 were stimulated with the WT NP₃₃₈ peptide for 10 days. WT NP₃₃₈-specific CD8⁺ T cell lines were tetramer stained individually with each of the variant NP₃₃₈ tetramers conjugated to PE. Following surface staining, lymphocytes, singlets, live, CD3^{mid-high} tetramer⁺ CD8⁺ T cells were single cell-sorted and the TCRαβ repertoire was determined using a multiplex RT-PCR. **(a)** Graphical representation and **(b)** summary of the TCRαβ repertoire used by a WT NP₃₃₈-specific CD8⁺ T cell line for the recognition of the variant NP₃₃₈ peptides. **(c)** Summary of CDR3α and CDR3β length used by CD8⁺ T cells for the recognition of NP₃₃₈ peptides.



Supplementary Figure 2. Gating strategies used for this study

(a) Gating strategy used for ICS of epitope-specific CD8⁺ T cell lines as used in Figures 1 and 2. **(b)** Gating strategy used for tetramer staining of epitope-specific CD8⁺ T cell lines as used in Figure 2 and Supplementary Figure 1. * FACS plot shown was not from a sorted sample and is indicative of the gating strategy only. **(c)** Gating strategy used for sorting of epitope-specific CD8⁺ T cells directly ex vivo following magnetic enrichment as used in Figures 4, 5 and 8.

Supplementary Table 1. Thermal stability of pH_A complexes

HLA-peptide complex	Tm (°C)
HLA-B*37:01-NP ₃₃₈	61.6 ± 0.8
HLA-B*37:01-NP ₃₃₈ -L7S	61.7 ± 1.2
HLA-B*37:01-NP ₃₃₈ -V6L	57.6 ± 1.1
HLA-B*18:01-NP ₃₃₈	55.7 ± 1.2
HLA-B*44:05-NP ₃₃₈	47.5 ± 0.5

Tm, or thermal melt, is the temperature required to reach 50% of unfolded protein.

Supplementary Table 2. Data Collection and Refinement Statistics

Data Collection Statistics	HLA-B*37:01-NP ₃₃₈	HLA-B*37:01-NP ₃₃₈ -L7S	HLA-B*37:01-NP ₃₃₈ V6L	HLA-B*18:01-NP ₃₃₈	HLA-B*44:05-NP ₃₃₈	EM2 TCR-HLA-B*37:01-NP ₃₃₈
Space group	P 2 ₁ 2 ₁ 2 ₁					
Cell Dimensions (a,b,c) (Å)	50.88, 81.79, 110.19	50.97, 81.72, 110.56	50.86, 82.04, 110.20	50.83, 81.55, 110.69	82.19, 110.52, 50.90	46.81, 97.92, 185.59
Resolution (Å)	46.19 – 1.31 (1.33 – 1.31)	81.72 – 1.50 (1.58 – 1.50)	82.04 – 1.55 (1.63 – 1.55)	46.19 – 1.21 (1.23 – 1.21)	46.23 – 1.35 (1.42 – 1.35)	47.40 – 3.00 (3.16 – 3.00)
Total No. of obs.	802119 (113954)	507353 (78130)	453643 (65126)	984959 (40588)	696389 (93213)	131903 (19319)
No. of unique obs.	111938 (16061)	73354 (10768)	67762 (9785)	140506 (6636)	101079 (13835)	18278 (2614)
Multiplicity	7.2 (6.9)	6.9 (7.3)	6.7 (6.7)	7.0 (6.1)	6.9 (6.7)	7.2 (7.4)
Data completeness (%)	99.8 (99.1)	98.3 (100.0)	100.0 (100.0)	99.6 (95.8)	98.8 (93.9)	100.0 (100.0)
I/σ _I	13.3 (3.4)	6.9 (2.3)	9.7 (2.1)	11.8 (2.4)	10.8 (2.4)	6.3 (2.0)
R _{pim} ^a (%)	3.7 (30.5)	6.6 (30.2)	8.0 (42.8)	3.8 (30.5)	3.8 (30.4)	13.4 (39.1)
Refinement Statistics						
R _{factor} ^b (%)	17.17	21.92	18.27	19.20	19.17	21.84
R _{free} ^b (%)	19.27	25.06	20.67	21.36	21.05	30.12
rmsd from ideality						
Bond lengths (Å)	0.010	0.010	0.005	0.010	0.010	0.008
Bond angles (°)	1.02	1.04	0.97	1.06	1.03	1.03
Ramachandran plot (%)						
Allowed region	98.5	98.2	98.2	98.5	98.4	90.4
Disallowed region	0	0.2	0.80	0	0	1.8

^aR_{p.i.m} = $\sum_{hkl} [1/(N-1)]^{1/2} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \langle I_{hkl} \rangle$. ^bR_{factor} = $\sum_{hkl} ||F_o|| - ||F_c|| / \sum_{hkl} |F_o|$ for all data except $\approx 5\%$ which were used for R_{free} calculation. Values in parentheses are for the highest resolution-shell.

Supplementary Table 3. Donors used in this study and their HLA profiles

	Donor	HLA-A	HLA-B
HLA-B*37:01	Donor 1	01:01, 23:01	40, 37:01
	Donor 2	01:25, 11:01	13:02, 37:01
	Donor 3	01:01, 68:01	35:03, 37:01
	Donor 4	01:01, 01:01	08:01, 37:01
	Donor 5	01:01, 24:02	08:01, 37:01
HLA-B*18:01	Donor 6	02:05, 25	18:01 44:02
	Donor 7	29:02, 30:02	18:01, 44:03
	Donor 8	02:01, 03:02	18:01, 35:08
	Donor 9	02, 25:01	07:02, 18:01
	Donor 10	02:01, 02:01	15, 18:01
HLA-B*44:02	Donor 11	02:01, 11:01	35:03, 44:02
	Donor 12	02:01, 29:02	140:2, 44:02
	Donor 13	03:01, 32:01	44:02, 44:02
	Donor 14	03:01, 24:02	35:03, 44:02
	Donor 15	02:01, 02:01	07:02, 44:02
HLA-B*44:03	Donor 16	03:01, 11:01	07:02, 44:03
	Donor 7	29:02, 30:02	18:01, 44:03
	Donor 17	02:01, 32:01	07:02, 44:03
	Donor 18	01:01, 26:01	08:01, 44:03
	Donor 19	02, 24:02	13:02, 44:03
HLA-A*01:01	Donor 1	01:01, 23:01	40, 37:01
	Donor 20	01:01, 24:02	08:01, 57:01
	Donor 21	01:01, 31:01	07:02, 35:01

Alleles of interest are shown in bold.

Supplementary Table 4. TCR $\alpha\beta$ repertoire usage for the recognition of NP₃₃₈-variant peptides

Code	TRAV	TRAJ	CDR3 α	Length	TRBV	TRBJ	CDR3 β	Length	Frequency					
									D1			D2		
									WT	L7S	V6L	WT	L7S	V6L
AA	14	43	CAMRGSGMNNDMRF	9	20-1	1-6	CSARAYRGYNPLHF	10	16	38	4			
AB	29	43	CAARGGNNDMRF	6	27	1-1	CASSLTGQGAFF	7	16	23	4			
AC	21	47	CAAPLEYGNKLVF	8	27	2-6	CASGRRLVTGGGTGANVLTF	14	8	8				
AD	3	26	CAVRLTRDNYGQNFVF	11	14	2-1	CASSQVDYNEQFFF	8	4		7			
AE	3	37	CAVRDSASGNTGKLF	11	19	1-5	CASSWGTGGNQNPQHF	10	4		4			
AF	6	27	CAHVNTNAGKSTF	8	5-5	1-3	CASSGLINGNTIYF	9	4		19			
AG	12-2	21	CAVKGRYFNFKFYF	9	19	2-7	CASSVGTGAYEQYF	9	4		4			
AH	19	50	CALSNAKTSYDKVIF	10	20-1	1-5	CSARPGQFYSNQNPQHF	11	4		11			
AI	17	45	CATDFALSSGGGADGLTF	13	3	1-5	CASSLTGHNQNPQHF	9		19	7			
AJ	8-3	24	CALTDSWGKFQF	7	11-2	2-5	CASTGTSGRAETQYF	10		8				
AK	3	34	CAVRDGGVLRDTKLF	11	19	2-7	CASSISSGRTYEQYF	10		4				
AL	3	34	CAVRGVRSYNTDKLF	11	19	2-3	CATQGTSGYTDTQYF	10		4				
AM	8-1	48	CAVKGVNFGNEKLF	10	7-2*	2-2	CASNWGGRGNTGELFF	11		4				
AN	13-2	15	CAENRLGTALIF	7	27	1-1	CASSSTGREAFF	7		4				
AO	21	28	CAVLHGAGSYQLTF	9	27	2-2	CASSQTGRELFF	7		4				
AP	21	28	CAVLSGAGSYQLTF	9	27	2-7	CASSPTQREQYF	7		4				
AQ	26-1	24	CIVRVPGLGDSWGKLOF	12	7-2	1-5	CASSWGWRSNQNPQHF	10		4				
AR	26-1	22	CIVRVGTSGSARQLTF	11	19	1-2	CASSISPSGFNGYTF	10		4				
AS	13-1	58	CAASHETSGSRLTF	9	15	1-6	CATSRDGLAGANSPLHF	12		4				
AT	21	30	CAVYRDDKIIF	6	11-2	2-7	CASSLWGRALSYEQYF	11		4				
AU	38-1	57	CAYRSATRSSLISEKLVF	13	27	1-6	CASRTSSYNSPLHF	9		4				
AV	21	15	CAVSPVNQAGTALIF	10	19	2-1	CASSLGRGVQFF	7		7				
AW	30	37	CGTERRPSNTGKLF	10	19	2-5	CASSIPDGTQYF	7		7				
AX	3	34	CAVSVRADKLIF	7	29-1	2-1	CSVEGLAGPSYNEQFF	11		4				
AY	12-2	47	CAVNIRGPMEYGNKLVF	13	19	1-2	CASSMLVANYGYTF	9		4				
AZ	17	45	CATDAVQSTGGGADGLTF	13	3	1-5	CASSRTGRNQNPQHF	9		4				
BA	21	28	CAVLRGAGSYQLTF	9	27	2-2	CASSPTAGELF	7		4				
BB	23	34	CAARGRYNTDKLF	9	19	2-7	CASSILQGRSYEQYF	10		4				
BC	13	11	CAASGPAGYSTLTF	9	19	1-1	CASSIVQRTNTEAFF	10		4				
BD	36	28	CAVGTRLDGAGSYQLTF	12	19	1-1	CASTLGTVEAFF	7		4				
	38-1	35	CAFMRGFGNVLHC	8										
CA	35	41	CAGRNMNSGYALNF	9	19	1-1	CASSIGVRAFF	6		42	5	14		
CB	3	9	CAVSYTGGFKTIF	8	19	1-6	CASSMAQGARSPLHF	10		17		19		
CC	19	39	CALSAHRHENAGNMLTF	12	20	1-5	CSARDGQVYSNQNPQHF	11		4		10		
CD	20	26	CAVLLPRDNYGQNFVF	11	12	2-1	CASSLAVHNEQFF	8		4		10		
CE	19	15	CALSAGGLDQAGTALIF	12	19	1-2	CASSMGRDGYTF	7		4		5		
CF	19	10	CALSGWGLTGGGNKLTF	12	4	2-3	CASSPGTAADTQYF	9		8				
CG	12-3	43	CAMSPSFMANDMRF	9	9	1-1	CASSEGTTGGLNAFF	9		4				
CH	21	28	CAVLEGAGSYQLTF	9	27	2-1	CASSLTQNEQFF	7		4				
CI	21	32	CAVRSEWEAGTNKLIF	11	11-2	2-1	CASSALTGNEQFF	8		4				
CJ	29	41	CAASANSGYALNF	8	27	1-5	CASSSLRVGAGPGQQPQHF	13		4				
CK	36	39	CAVGSSYPNAGNMLTF	10	4-1	1-5	CASSQEAGAYNQNPQHF	11		4				
CL	21	58	CAVLGRTSGSRLTF	9	4-1	1-5	CASSQDMGGYNQNPQHF	11		24				
CM	22	27	CAVEPTNAGKSTF	8	4-1	1-5	CASSQDQGAYNQNPQHF	11		19				

CN	41	43	CAAPSNDMRF	5	4-1	1-6	CASSQDLGSYNSPLF	11		14		
CO	1-2	13	CAVRVPGYQKVTF	8	4	2-1	CASSQDPGQGYNEQFF	11		5		
CP	3	22	CAVRDGFWSARQLTF	11	14	2-3	CASSQGPTTDTQYF	9		5		
CQ	8	5	CAVRVLDTGRRALTF	10	19	1-2	CASSPYTDRQENYGYTF	12		5		
CR	12-2	22	CAPGMASGSARQLTF	10	20-1	1-6	CSAREPGRQFAAHLHF	12		5		
CS	17	50	CATPPKTSYDKVIF	9	27	1-6	CASSLLKGTLHF	7		5		
CT	21	27	CAVLPNTNAGKSTF	9	4-1	1-5	CASSQDAGLYNQPQHF	11		5		
CU	27	20	CAGPYSNDYKLSF	8	27	2-3	CASSIYRRRQGALAGPDTQYF	16		5		
CV	38-2	57	CAYRSADRLRRGSEKLVF	13	27	1-6	CASRPPHYNSPLF	9		5		
CW	17	57	CATVPALTQGGSEKLVF	12	27	2-6	CASSMLGVLRGANVLTF	12		10		
CX	38-1	28	CAFMKHRLNSGAGSYQLTF	14	27	2-2	CASSSLAVPELFF	8		10		
CY	26-1	42	CIVRVAGYGGSQGNLIF	12	19	1-6	CASSIGSGMNSPLHF	10		5		
CZ	8-6	6	CAVRDASGGSYIPTF	10	4-1	2-7	CASSLIEGLSEQYF	9		5		
DA	21	15	CAASSVRQAGTALIF	10	19	2-1	CASSIGRSEQFF	7		5		
DB	41	45	CAVKGSYSGGGADGLTF	12	7-2	2-2	CASSLRSGTGELFF	9		5		
DC	41	45	CAVRTGGADGLTF	9	7-2	2-2	CASSQRSGTGELFF	9		5		
	29	28	CAAISPGAGSYQLTF	10								
EA	23	52	CAATYTAGGTSYKLTF	12	27	1-2	CASTNYAGGDGRGYTF	10		11	38	12
EB	21	28	CAGLRGAGSYQLTF	9	27	2-4	CASSATKNIQYF	7		5	8	6
EC	14	4	CAMREVSGGYNKLIF	10	9	2-3	CASSVVATGGRGTDTQYF	13		5	8	
ED	8-3	34	CAVGARETYNTDKLIF	11	19	1-6	CASSPRQGNNSPLHF	10		11		6
EE	30	13	CGTERSGGYQKVTF	9	19	1-1	CASSMSAMGTEAFF	9		16		35
EF	9-2	24	CALRTDSWKGKLF	8	12-5	2-5	CASGPTGGAETQYF	10		21		
EG	12-2	24	CAVTIGWGKLF	7	29-1	1-1	CSVYLNTEAFF	6		5		
EH	12-3	37	CAMSALRSPSNTGKLIF	12	19	2-5	CASSMGTGPRETQYF	10		5		
EI	12-3	21	CAMKGPYNFNKFYF	9	19	1-2	CASSIGTGGNYGYTF	10		5		
EJ	13-1	11	CAATLGSGYSTLTF	9	19	1-5	CASSMGTYSNQPQHF	10		5		
EK	35	58	CAGQLPPWETSGSRLTF	12	19	1-6	CASRLQGANSPLF	10		5		
EL	19	33	CALSEAGSNYQLIW	9	5-6	1-1	CASSLGTPTEAFF	8		5		
	13-1	9	CAARGGFKTIF	6								
EM	36	22	CAVTGLSGSARQLTF	11	27	2-1	CASSPTRLVSSYNEOFF	12		8		
	23	40	CAARKATPSGTYKYIF	11								
EN	34	40	CGAVLSGTYKYIF	8	5-8	2-7	CASSLGPTTYKSSYEQYF	13		8		
EO	17	45	CATVPLLTSGGGADGLTF	13	3	1-5	CASSPTGRVQPQHF	9		8		
EP	4	5	CLVGHFMDTGRRALTF	11	27	2-5	CASRSLAEKGETQYF	10		4		
EQ	21	28	CAVLEGAGSYQLTF	9	27	1-1	CASSLAQGKAFF	7		4		
ER	29	32	CAASEYGGATNKLIF	10	27	1-6	CASSPLHLDGPKTPLHF	13		4		
ES	30	57	CGTITQGGSEKLVF	9	9	2-1	CASSVGLAEPLDEQFF	11		4		
ET	30	49	CGTENTGNQFYF	7	11-2	2-5	CASSLGPGBKSSKTQYF	11		4		
EU	21	17	CAVLPKAAGNKLTF	10	19	1-1	CASSGVRATEAFF	8		24		
EV	14	49	CAMREGGNQFYF	7	11-2	2-1	CASSLPGPNHNEQFF	10		6		
EW	20	30	CAVQAMGDKIIF	7	11-2	2-1	CASSLLAGYNEQFF	9		6		
EX	27	21	CAGENTYNFNKFYF	9	14	1-2	CASSQLPRGGYTF	8		6		
							# of sequences	25	26	27	24	21
							Frequency of cross-reactivity	60	88	59	71	5
								57	47	54	59	

TRAV represents the variable α-chain gene usage; TRBV represents the β-chain gene usage; frequencies in red represent universal cross-reactive TCRαβ clonotypes whilst other cross-reactive TCRαβ clonotypes are in blue. Each clonotype frequency is

expressed as the proportion of total sequences. The EM2 clonotype, from Donor 4, is shaded in blue.

Supplementary Table 5. Contact table of the EM2 TCR with HLA-B37-NP₃₃₈

TCR gene	TCR residue	MHC residue	Bond type
CDR1 α	Tyr30	Glu154, Gln155, Ala158	VDW
CDR2 α	Leu57	Arg151, Glu154	VDW
CDR2 α	Lys58	Arg151	VDW
CDR3 α	Gly111	Arg62	VDW
CDR3 α	Tyr112-OH	Glu58-O ϵ 1, Arg62	VDW, HB
CDR1 β	Asn28	Lys146	VDW
CDR1 β	Asp30	Glu76	VDW
CDR2 β	Gln57	Thr69	VDW
CDR2 β	Ile58	Thr69, Gln72, Thr73, Glu76	VDW
CDR2 β	Val59	Gln72, Glu76, Arg79	VDW
CDR2 β	Asn60-N δ 2	Gln72-O ϵ 1, Arg75	VDW, HB
CDR2 β	Asp61-O δ 2-O δ 1	Lys68-O, Thr69-O γ 1	VDW, HB
FW β	Gln67	Gln65	VDW
FW β	Lys83-N ζ	Glu76-O ϵ 1-O ϵ 2	VDW, SB
CDR3 β	Met109	Lys146	VDW
CDR3 β	Ser110	Gln155	VDW
CDR3 β	Met112-O	Gln155-N ϵ 2-O ϵ 1	VDW, HB
CDR3 β	Gly113	Gln155	VDW
TCR gene	TCR residue	Peptide residue	Bond type
CDR3 α	Ser109	Asp3	VDW
CDR3 α	Gly110-N	Phe1, Asp3-O δ 1-O δ 2	VDW, HB
CDR3 α	Gln113-N ϵ 2-O ϵ 1	Asp3-O δ 1-O δ 2, Leu4	VDW, HB
CDR1 β	Asp30-O δ 1-O δ 2	Leu7, Ser8-O γ	VDW, HB
CDR2 β	Gln57	Leu4	VDW
CDR3 β	Met109	Leu7	VDW
CDR3 β	Ser110	Val6, Leu7	VDW

VDW: Van der Waals interaction (cut-off at 4 Å), HB: hydrogen bond (cut-off at 3.5 Å), SB: salt bridge (cut-off at 5 Å).

Supplementary Table 6. Surface plasmon resonance (SPR) of the EM2 TCR for NP₃₃₈ and variants

pHLA complex	K _{deq} (μ M)	k _{on} ($\times 10^4 M^{-1}s^{-1}$)	k _{off} (s ⁻¹)	K _{dcal} (μ M)
HLA-B*37:01-NP ₃₃₈	133.0 ± 9.0	ND	ND	ND
HLA-B*37:01-NP ₃₃₈ -L7S	150.5 ± 14.5	ND	ND	ND
HLA-B*37:01-NP ₃₃₈ -V6L	32.3 ± 0.2	1.046 ± 0.0064	0.214 ± 0.004	20.8 ± 1.5
HLA-B*18:01-NP ₃₃₈	NB	ND	ND	ND
HLA-B*44:05-NP ₃₃₈	NB	ND	ND	ND

The equilibrium dissociation constant (K_{d_{eq}}) values represent the mean ± sem (standard error from the mean) from at least two independent experiments carried in duplicate. NB, no binding observed at the maximum concentration tested; ND, not determined.

Supplementary Table 7. Conservation of NP₄₄ epitope in distinct IAV strains

Sequence	Abbreviation	IAV - H1N1			IAV - H3N2			Other IAV Strains		
		All	Aust	Vacc	All	Aust	Vacc	pH1N1 All	H5N1 All	H7N9 All
CTELKLSDY	WT	79.7	67.4	33.3	34.5	35.2	75.0	98.3	84.5	
CTELKLNDY	S7N	18.3	23.3	66.7	0.2		6.3	0.9		
CTELKLSDH	Y9H	0.1			63.8	64.2	18.8			
CTELKLSEY	D8E	0.3						0.3		
CTELKLTDY	S7T	0.1			0.1			0.1		
CTELRLSDY	K5R	0.2						0.2		
CTGLKLSDY	E3G	0.1						0.1		
CTELKLDHY	S7D	0.1								
CTELKLRDY	S7R	0.1								
CTELKLSNY	D8N	0.1								
CTELQLSDY	K5Q	0.3			0.2					
CTEIKLNDY	L4I + S7N	0.1								
CTELKLNHDH	S7N + Y9H	0.5	9.3		0.1					
CTEELSDY	K5E				0.1					
CTELKLSDC	Y9C				0.1					
CTEIKLSDY	L4I							1.0		
CTELKLSDN	Y9N				0.1			1.0	94.9	
CTELKLSDQ	Y9Q				0.1			12.4		
CTELKLTDH	S7T + Y9H				0.2	0.6				
CTEIKLSDH	L4I + Y9H				0.2					
CTELKFSDH	L6F + Y9H				0.2					
CTELKHSDH	L6H + Y9H				0.1					
CTELKLGDH	S7G + Y9H				0.2					
CTELKLIIDH	S7I + Y9H				0.1					
CTELKLSGH	D8G + Y9H				0.1					
CTELKLTDO	S7T + Y9Q							1.0		
CTELKLSDS	Y9S								2.6	
CTELKLNNDN	S7N + Y9N								2.6	
<i>Number of sequences</i>		1155	43	9	126 4	159	16	858	97	39
<i>Coverage (%)</i>		98.0	90.7	100.0	34.6	35.2	81.3	99.2	84.5	0.0

Sequences were obtained from the NCBI Influenza Research Database <https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database>. Full-length sequences of Australian (denoted Aust), vaccine (denoted Vacc) and pH1N1, H5N1 and H7N9 were obtained and were aligned using the influenza database. Underlined are anchor residues for HLA-B*37:01, and mutations are shown in bold. Shaded rows represent sequences chosen for further analysis.

Supplementary Table 8. TCR $\alpha\beta$ repertoire usage for the recognition of NP₄₄-variant peptides

Code	TRAV	TRAJ	CDR3 α	TRBV	TRBJ	CDR3 β	Frequency				
							D1		D20	D21	
							WT	S7N	WT	S7N	WT
GA	8-2	21	CVV-SDRNFNKF-YF	10-3	2-3	CAI-SENGADTQ-YF	14	16			
GB	8-2	2-5	CVV-SDRNFNKF-YF	10-3	2-5	CAI-SESQGPGGETQ-YF	3	34			
GC	29	34	CAA-SGRTDKL-IF	9	2-1	CAS-SVESSGSPYQ-FF	3	3			
GD	39	57	CAV-DSWAGGSEKL-VF	25-1	1-2	CAS-SERGDTDLGY-TF	10				
GE	3	15	CAL-DQAGTAL-IF	3-	2-3	CAS-RPLSGGAPDTQ-YF	10				
GF	41	49	CAV-RLLLRNQF-YF	9	2.7	CAS-SVGAGPTYEQ-YF	3				
GG	41	49	CAVRFLGNQFYF	9	2.7	CASSVGAGPSYEQYF	3				
GH	4	30	CLV-GVRDDKI-IF	10-3	2-1	CAT-KPPGTDEQ-FF	3				
GI	5	12	CAE-TTDSSYKL-IF	28	2-7	CAS-TRQGDSYEQ-YF	3				
GJ	12-1	42	CVV-TGVHYGGSQGNL-IF	2	2-7	CAS-SESGSPSYEQ-YF	3				
GK	12-1	39	CVV-TSGML-TF	7-6	2-2	CAS-SPGTDYTGEL-FF	3				
GL	12-3	17	CAM-IKAAGNKL-TF	27-1	1-1	CAS-SINRASEA-FF	3				
GM	13-1	35	CAA-GRKRGFGNVL-HC	15	2-5	CAT-SREW PANRHQETQ-YF	3				
GN	13-1	4	CAA-TFSSGGYNKL-IF	6-5	2-1	CAS-RSGGANEQ-FF	3				
GO	13-2	23	CAE-ILYNQGGKL-IF	24-1	1.2	CAT-SDFNHGY-TF	3				
GP	14	37	CAM-REGTGKL-IF	11-2	1-2	CAS-SLEAAPGRYGY-TF	3				
GQ	19	23	CAL-MRNQGGKL-IF	5-8	2-1	CAS-SLEPQDTQ-YF	3				
GR	25	15	CAG-PNQAGTAL-IF	7-3	2-1	CAS-SLTGTGSYNEQ-FF	3				
GS	26-2	37	CIR-VGSNTGKL-IF	5-8	1-1	CAS-SLDSPPGVTEA-FF	3				
GT	29	54	CAA-SRGAQKL-VF	4-1	1-4	CAS-SHLQGSEKL-FF	3				
GU	29	41	CAA-SGYAL-NF	12	2-3	CAS-SFGGRAGD TDQ-YF	3				
GV	38-2	34	CAY-QVHTDKL-IF	16	1-1	CAS-SQEDGFTEA-FF	3				
GW	8-2	21	CVV-SDRNFNKF-YF	10-3	2-3	CAI-SESLGADTQ-YF		9			
GX	8-2	21	CVV-SDRNFNKF-YF	10-3	2-3	CAG-RDHTDTQ-YF		9			
GY	12-2	38	CAG-GAGNNRKL-IW	4-1	1-5	CAS-SQDGISTGDQPQ-HF		9			
GZ	8-2	21	CVV-SDRNFNKF-YF	10-3	1-2	CAI-SEE GTGFYGY-TF		6			
HA	24	6	CAP-ASGGSYIP-TF	10-3	2-7	CAI-GGDGSYEQ-YF		6			
HB	8-2	21	CVV-SDRNFNKF-YF	10-3	2-5	CAI-SEFQGPGGETQ-YF		3			
HC	14	11	CAM-REESGYSTL-TF	4	1-1	CAS-SQDGAGTPFTEA-FF		3			
HD	1-2	33	CAV-VDSNYQL-IW	3	2-1	CAS-SQE GEAHNEQ-FF			5		
HE	1-2	33	CAV-RDSNYQL-IW	6-4	2-1	CAS-SDSPGVGYNEQ-FF			5		
HF	1-2	20	CAV-KDRDYKL-SF	6-4	1-5	CAS-SEGENQPQ-HF			5		
HG	1-2	33	CVL-MDSNYQL-IW	20-1	1-1	CSA-RRADTE-AFF			5		
HH	1-2	33	CAV-MDSNYQL-IW	20-1	2-7	CSA-REHREP YEQ-YF			5		
HI	1-2	33	CAV-LDSNYQL-IW	20-1	2-3	CSA-RGTSGETY TQ-YF			5		
HJ	1-2	33	CAV-TDSNYQL-IW	20-1	1-1	CSA-RTGDRTEA-FX			5		
HK	1-2	33	CVP-MDSNYQL-IW	24-1	2-2	CAT-SDRDRDTGEL-FF			5		
HL	1-2	33	CAV-KDSNYQL-IW	28	2-6	CAS-SFGRLSGANVL-TF			5		
HM	3	30	CAV-TPGDKI-IF	5-3	1-1	CAS-SFSVRDMGTEA-FF			5		
HN	6	34	CAL-DTDKL-IF	28	2-3	CAS-SLWASGKWSTDQ-YF			5		

HO	12-2	3	CAV-NMARYSSASKI-IF	19	2-2	CAS-NPPGLAATGEL-FF	5	
HP	12-3	30	CAI-GDDKI-IF	28	2-3	CAS-SSTSGRARDTQ-YF	5	
HQ	12-3	36	CAM-EGGANNL-FF	19	1-2	CAS-TSVMTDTSIYGY-TF	5	
HR	13-2	26	CAE-KKDNYGQNF-VF	6-8	2-1	CAS-SYLGTSAPIDEQ-FF	5	
HS	19	58	CALSSKETSGSRLTF	4-2	1-5	CAS-SRTGEHQPO-HF	5	
HT	19	45	CAL-SDPYSGGGADGL-TF	7-9	2-5	CAS-SLAGGTGETQ-YF	5	
HU	19	11	CAL-SDGYSTL-TF	27	2-6	CAS-SLPGANVL-TF	5	
HV	19	49	CAL-SLDGNQF-YF	27	2-1	CAS-RLGESSIONEQ-FF	5	
HW	29	35	CAA-SERLGFGNVL-HC	5	2-2	CAS-SFLTGEL-FF	5	
HX	6	20	CAL-DGDYKL-SF	3	1	CAS-SQDNPYGANTEA-FF	59	
HY	8	21	CVV-SDRNFNKF-YF	10-3	2-7	CAI-SESIGLDEQ-YF	24	
HZ	8	21	CVV-SDRNFNKF-YF	10-3	2-7	CAI-SGWTGEGSEQ-YF	6	
IA	1-2	33	CAV-RNSNYQL-IW	6-4	2-3	CAS-SATGDATDTQ-YF	6	
IB	19	12	CAL-EMDSSYKL-IF	20	-5	CSA-GGLAGEETQ-YF	6	
IC	1-2	33	CAV-NNSRL-IW	20-1	2-7	CSA-RMVSYEQ-YF	5	
ID	3	16	CAV-RLLDGQKL-LF	7-6	2-1	CAS-SRGTYNEQ-FF	5	
IE	4	31	CLV-GDGARL-MF	28	2-7	CAS-SSSGYEQ-YF	5	
IF	5	23	CAE-TPQGGKL-IF	9	2-7	CAS-SVGRGSGEQY-F	5	
IG	8-6	43	CAG-GGHNNNDM-RF	30	2-7	CAY-LTGFEQ-YF	5	
IH	13-2	26	CAV-SVYVGQNF-VF	30	2-3	CAW-SPTSGRDTQ-YF	5	
II	17	45	CAT-ALYSGGGADGL-TF	19	1-1	CAS-SILGWSEA-FX	5	
IJ	19	31	CA-LSEARL-MF	4	2-1	CAS-SHLLAAYNEQ-FF	5	
IK	19	39	CAR-DNAGNML-TF	6-6	2-1	CAS-SYSAWNEQ-FF	5	
IL	19	45	CAL-TELGGGADGL-TF	30	2-1	CAW-SALAGGPSNEQ-FF	5	
IM	20	39	CAV-QARNNAGNML-TF	19	2-7	CAS-SIVKLAGGTYEQ-YF	5	
IN	21	54	CAV-WIQGAQKL-VF	11-3	2-3	CASS-LRTRTDTQ-YX	5	
IO	22	32	CAV-YSRGATNKL-IF	6-1	1-5	CAS-SVGRDSSGNQPQ-HF	5	
IP	25	42	CAG-SIMNYGGSQGNL-IF	9	2-7	CAS-SVSSGLYYEQ-YF	5	
IQ	27	42	CAG-DLSYGGSQGNL-IF	2	2-7	CAS-SWTSGRPEQ-YF	5	
IR	29	45	CAA-TGGGADGL-TF	9	2-7	CAS-SGASYEQ-YF	5	
IS	35	45	CAG-YSGGGADGL-TF	5-8	2-5	CAS-SPMTGDGTQ-YF	5	
IT	38-1	54	CAF-LPGGAQKL-VF	25-1	2-3	CAS-SVRADTQ-YF	5	
IU	41	53	CAV-SSFNSGGSNYKL-TF	6-4	2-1	CAS-SDSSFGVGLGNEQ-FF	5	
<i># of sequences</i>							29	32
<i># of sequences</i>							20	17
<i># of sequences</i>							19	

TRAV represents the variable α -chain gene usage; TRBV represents the β -chain gene usage; sequences in blue residues represent conserved CDR3 α chains. Each clonotype frequency is expresses as the proportion of total sequences.

Supplementary Table 9. Primers used in this study

TRAV	Primer sequence 5'-3'	
	External	Internal
1	AACTGCACGTACCAGACATC	GCACCCACATTCTKCTTAC
2	GATGTGCACCAAGACTCC	CACTCTGTGTCCTAACATGCTTAC
3	AAGATCAGGTCAACGTTGC	ATGCACCTATTCACTCTCTGG
4	CTCCATGGACTCATATGAAGG	ATTATATCACGTGGTACCAACAG
5	CTTTCCCTGAGTGTCCGAG	TACACAGACAGCTCCTCCAC
6	CACCCTGACCTGCAACTATAC	TGGTACCGACAAGATCCAG
7	AGCTGCACGTACTCTGTCAG	ACAATTGCACTGGTACAGG
8-1	CTCACTGGAGTTGGGATG	GTCAACACCTTCAGCTTCTC
8-3	CACTGTCTCTGAAGGAGCC	TTTGAGGCTGAATTAAAGAGG
8-2 & 4	GCCACCCTGGTAAAGG	AGAGTGAAACCTCCTCCAC
8-6	GAGCTGAGGTGCAACTACTC	AACCAAGGACTCCAGCTTC
8-7	CTCTGGAACTGAAGTGCAAC	GGAGTTCCTCTCTCTCTGG
9-1 & 2	TGGTATGTCCAATATCCTGG	GAAACCACTCTTCCACTTG
10	CAAGTGGAGCAGAGTCCTC	GAAAGAACTGCACCTCAATG
12-1 & 3	CARTGTTCCAGAGGGAGC	AAGATGGAAGGTTACAGCAC
13-1	CATCCTCAACCCCTGAGTG	TCAGACAGTGCCTCAAACATAC
13-2	CAGCGCCTCAGACTACTTC	CAGTGAAACATCTCTCTGC
14	AAGATAACTCAAACCCAACCAG	AGGCTGTGACTCTGGACTG
16	AGTGGAGCTGAAGTGCAC	GTCCAGTACTCCAGACAACG
17	GGAGAAGAGGATCCTCAGG	CCACCATGAACTGCAGTTAC
18	AGAAAACCAGGAGACGGAC	CAGGCCAGTCCTATCAAGAG
19	AGGTAACTCAAGCGCAGAC	TGTGACCTTGGACTGTGTG
20	CACAGTCAGCGGTTAACAGAG	TCTGGTATAGGCAAGATCCTG
21	TTCCTGCAGCTCTGAGTG	AACTGGTTCTCAACTGCAG
22	GTCCTCCAGACCTGATTCTC	CTGACTCTGTGAACAATTGC
23	TGCTTATGAGAACACTGCG	TGCATTATTGATAGCCATACG
24	CTCAGTCACTGCATGTTAG	TGCCTTACACTGGTACAGATG
25	GGACTTCACCACGTACTGC	TATAAGCAAAGGCCTGGTG
26-1	GCAAACCTGCCTTGTAAATC	CGACAGATTCACTCCCAG
26-2	AGCCAAATTCAATGGAGAG	TTCACTTGCCTTGTAAACCAC
27	TCAGTTCTAACGCATCCAAGAG	CTCACTGTGACTGCAACTCC
29	GCAAGTTAACGAAAATTCACC	CTGCTGAAGGTCCTACATT
30	CAACAACCAGTGCAGAGTC	AGAAGCATGGTAAGCAC
34	AGAACTGGAGCAGAGTCCTC	ATCTCACCATAAAACTGCACG
35	GGTCAACAGCTGAATCAGAG	ACCTGGCTATGGTACAAGC
36	GAAGACAAGGTGGTACAAAGC	ATCTCTGGTTGTCCACGAG
38	GCACATATGACACCAGTGAG	CAGCAGGCAGATGATTCTC
39	CTGTTCCCTGAGCATGCAG	TCAACCACCTCAGACAGACTG
40	GCATCTGTGACTATGAACGTG	GGAGGGGAAATATTAAAGAC
41	AATGAAGTGGAGCAGAGTCC	TTGTTATGCTGAGCTCAGG
Constant	GACCAGCTTGACATCACAG	TGTTGCTCTGAAGTCCATAG
TRBV	External	Internal

2	TCGATGATCAATTCTCAGTTG	TTCACTCTGAAGATCCGGTC
3	CAAAATACCTGGTCACACAG	AATCTTCACATCAATTCCCTG
4	TCGCTTCTCACCTGAATG	CCTGCAGCCAGAACAGACTC
5-1 & 4	GATTCTCAGGKCKCCAGTT	CTTGGAGCTGGRSGACTC
5-5 & 8	GTACCAACAGGYCCTGGGT	TCTGAGCTGAATGTGAACG
6-1 & 3 & 5 & 9	ACTCAGACCCCCAAAATTCC	GTGTRCCCAGGATATGAACC
6-4	ACTGGCAAAGGAGAAGTCC	TGGTTATAGTGTCTCCAGAGC
7-1 & 3	TRTGATCCAATTTCAGGTCA	TCYACTCTGAMGWTCCAGCG
7-4 & 9	CGSWTCTYTGCAGARAGGC	TGRMGATYCAGCGCACA
9	GATCACAGCAACTGGACAG	GTACCAACAGAGCCTGGAC
10	TGTWCTGGTATCGACAAGACC	TCCYCCTCACTCTGGAGTC
11	CGATTTCCTGCAGAGACGC	GACTCCACTCTCAAGATCCA
12	ARGTGACAGARATGGGACAA	CYACTCTGARGATCCAGCC
13	AGCGATAAAGGAAGCATCC	CATTCTGAACTGAACATGAGC
14	CCAACAATCGATTCTTAGCTG	ATTCTACTCTGAAGGTGCAGC
15	AGTGACCCCTGAGTTGTTCTC	ATAACTTCCAATCCAGGAGG
16	GTCTTGATGAAACAGGTATGC	CTGTAGCCTTGAGATCCAGG
17	AGTTGCTGATTCCTTCCAG	CACGCTGAAGATCCATCC
18	CATAGATGAGTCAGGAATGCC	CGATTTCCTGCTGAATTCC
19	AGTTGTGAACAGAACATTGAACC	TTCCTCTCACTGTGACATCG
20	AAGTTTCTCATCAACCATGC	ACTCTGACAGTGACCAAGTGC
23	GCGATTCTCATCTCAATGC	GCAATCCTGTCCTCAGAAC
24	CCTACGGTTGATCTATTACTCC	GATGGATACAGTGTCTCTCGA
25	ACTACACCTCATCCACTATTCC	CAGAGAAGGGAGATCTTCC
27	TGGTATCGACAAGACCCAG	TTCYCCCTGATYCTGGAGTC
29	TTCTGGTACCGTCAGCAAC	TCTGACTGTGAGAACATGAG
30	TCCAGCTGCTCTTACTCC	AGAATCTCTCAGCCTCCAGAC
Constant	TAGAACTGGACTTGACAGCG	TTCTGATGGCTCAAACACAG
Vector	Sense	Anti-sense
	GTAAAACGACGGCCAG	CAGGAAACAGCTATGAC

TRAV represents the variable α -chain gene usage; TRBV represents the β -chain gene usage.